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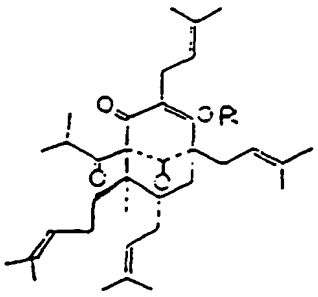

(I)

(57) Abstract

Hyperforin derivatives of formula (I), wherein R is an acyl or a glicoside group, have advantageous pharmacological characteristics and good stability.

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HYPERFORIN DERIVATIVES, THE USE THEREOF AND FORMULATIONS CONTAINING THEM

The present invention relates to novel hyperforin derivatives, the use thereof for the treatment of depression and anxiety, as well as the formulations containing them.

Flowering tops of Hypericum perforatum contain a number of classes of structurally different substances acting directly or indirectly on central nervous system.

More particularly, said compounds comprise hypericin, hyperforin, and dimeric flavones which exert antidepressive and anxiolytic activities on animals and humans.

The action mechanisms of these compounds are different: anti-MAO action, action on serotonin release, and benzodiazepine-like activity.

Hyperforin, which is one of the main components of the lipophilic fraction of Hypericum perforatum flowering tops, has recently been the object of numerous studies which made it possible to establish its important role as antidepressant; studies carried out by the Applicant proved that this molecule has serotonin-mimetic activity.

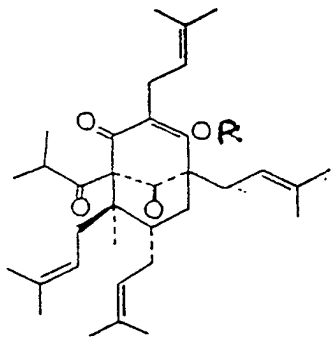
Hyperforin is not very stable in the usual conditions of extraction and conservation; according to WO 97/13489 (Schwabe), the hyperforin content in a water-alcoholic extract of St.-John's-wort falls nearly to zero already after a few weeks.

Again according to WO 97/13489, in order to obtain stable extracts with a constant content in hyperforin, extraction, purification and conservation should be

carried out in the presence of antioxidants such as vitamin C and the esters thereof, sulfated amino acids, etc.

The high instability of hyperforin makes therefore the preparation of formulations rather difficult.

Now hyperforin derivatives have been found which are stable and more active as antidepressants in specific pharmacological tests. The derivatives of the invention have the following formula I:



in which R is:

- a saturated or unsaturated, straight or branched, C_1-C_{22} acyl group, optionally having one or more substituents, which can be the same or different, selected from halogen atoms, nitro, amino, C_1-C_6-alkylamino, di-C_1-C_6-alkylamino, C_1-C_6-acylamino groups;
- a cycloaliphatic or aromatic acyl residue in which the aromatic moiety has optionally one or more substituents, which can be the same or different, selected from halogen atoms, hydroxy, methoxy, amino groups;
- a glycidic residue in which one or more hydroxy groups are optionally alkylated or acylated.

"Aromatic acyl residue" preferably means a benzoyl or cinnamyl residue having one or more amino or alkoxy

groups.

"Glycidic residue" means the residue of one sugar bound by an ether bond to the hydroxy group at the 1-position of the pyranosyl or furanosyl ring, being the other hydroxy groups of the sugar optionally methylated or acetylated.

Preferred R groups are acetyl, monochloroacetyl, butyryl, γ-aminobutyryl, p-aminobenzyl, trimethoxybenxyl, trimethoxycinnamyl, ß-glucosyl and ß-galactosyl.

The hyperforin derivatives of the invention can be prepared with conventional methods for the acylation or glicosylation of hydroxy groups.

For example, hyperforin substantially pure or an extract enriched in hyperforin can be subjected to reaction with acid chlorides or anhydrides of RCOOH acids (R as defined above) in suitable solvents, such as pyridine.

On the other hand, glicosylation can be carried out using a suitably protected reactive derivative of the desired sugar (ROH), for example α-D-glucopyranosyl bromide tetraacetate.

According to a particularly convenient aspect of the invention, compounds I are prepared by extracting Hypericum perforatum flowering tops with carbon dioxide in supercritical conditions, subsequently partitioning between solvents and derivatizing hyperforin in the resulting extract.

Leaves and flowering tops of St.-John's-wort, separately or in mixture, mainly as natural mixture, are extracted with carbon dioxide in supercritical conditions under pressures ranging from 180 to 260 bars,

preferably 240 bars and at temperatures ranging from 35 to 50°C, preferably 40°C. A lipophilic extract is obtained containing about 50% of hyperforin. The extract contains considerable amounts of xanthones, waxes, fatty acids and triglycerids. The Hyperforin percentage is subsequently increased, according to a further aspect of the invention, solubilizing the resulting extract in methanol or in partially aqueous acetonitrile and extracting then the solution with n-hexane or aliphatic hydrocarbons. The hydrocarbon phase contains undesired substances which are removed; the hydrophilic phase is diluted with about an equal volume of water and with aliphatic hydrocarbons. The extract of St.-John's-wort obtained by concentration of the lipophilic phase can be used for the preparation of the derivatives as described above.

The derivatives of the invention exert no activity *in vitro* on receptors, while being particularly active *in vivo*, exerting a dose-related strong antidepressive activity.

In an *in vivo* test in mice and rats, the compounds of the invention have shown a higher activity than hyperforin and Hypericum ethanolic or methanolic extracts.

As *in vivo* test to verify the antidepressive effect, were selected the escape deficit development test and the inhibition of the ethanol consumption in Sardinia alcohol preferring rats according to models known in literature.

In the escape deficit development test, the compounds of the invention have shown a higher activity than the known extracts and an activity comparable with

that of known medicaments such as imipramine. In this test, rats are fastened and subjected to mild, short, unavoidable electric shocks for 50 min (pre-test). Twenty-four hours later, animals are tested for their ability to avoid the same stimuli on their tails, in a situation in which escape is impossible. A rat on the average makes 26 escapes out of 30 stimuli (naive controls), whereas an animal subjected to pre-test only makes 1-3 escapes (ED controls). Hyporeactivity induced by the pre-test does not take place in rats pre-treated for 1-3 weeks with antidepressants such as imipramine or fluoxetine. The compounds of the invention administered to rats one hour before exposure to the unavoidable stress cause an increase in reactivity to the escape test, which is enhanced when pre-treatment is effected for 1-2 weeks.

For example, treatment of rats with compound I in which R is acetyl yields the results reported in the following Table:

Table - Antidepressive effect of Hyperforin the acetate in rats in rats in the escape test with a 2 week- pre-test.

Substances	Dose mg/kg	Number of escapes
Hyperforin acetate	6.25	12.6 ± 2.8
Hyperforin acetate	12.5	17.3 ± 1.9
Hyperforin acetate	25.0	21.2 ± 1.3
Hyperico alcoholic extract	1000	15.6 ± 2.4
Hyperico hexane extract	600	16.9 ± 1.2
Ed controls		2.6 ± 0.7
Naive controls		23.6 ± 1.2

Statistical analysis: Kruskal-Wallis non parametric ANOVA KW = 13.462 p = 0.0012

Hypericum alcoholic extract and hexane extract vs naive	p<0.01
Hyperforin acetate 25 mg vs naive	n.s.
Naive vs AND	p<0.01

In the test of the reduction of alcohol consumption in Sardinia rats (which is an index of depression and anxiety) according to procedures known in literature, the products of the invention induce, after two-day administration, a 60 to 75% decrease in alcohol consumption in favour of water compared with controls.

The compounds of formula I can be formulated in soft-gelatin capsules, hard-gelatin capsules, tablets, suppositories; preferably the compounds of the invention are formulated in soft-gelatin capsules or in controlled-release formulations. The dosages of compounds in the formulations range from 5 to 50 mg per dose in the usual formulations and up to 200 mg in the controlled-release formulations, in this case the preferred dose being 200 mg per dose daily.

The examples reported hereinbelow illustrate the invention in greater detail.

Example 1 - Preparation of a Hyperforin-enriched extract.

10 kg of biomass of *Hypericum perforatum* are extracted according to the procedure reported below, in a 25 L extraction plant for supercritical gas, equipped with two separators.

10 kg of *Hypericum perforatum* flowering tops mechanically dried, after collecting, at a temperature not above 60°C, are extruded into cubes so as to break

cells and extracted with CO_2 in supercritical conditions under the following experimental conditions:

- temperature: 45°C in the extractor, 30°C in the first separator and 20°C in the second separator;
- pressure: 240 bars in the extractor, 100 bars in the first separator and 50 bars in the second separator.

The CO_2 flow was 10 L per minute for 45 minutes. The extract was concentrated in the second separator, whereas most water present in the vegetable matrix was concentrated in the first separator. The extract present in the second separator is solubilised in 3.2 L of methanol and this solution is extracted with 3 x 1.5 L of n-hexane.

The hexane phase is counter-washed with 98% methanol using hyperforin as marker which should remain in the methanol phase. The hexane phase is removed whereas the combined metanolic ones are diluted with 0.6 L of water and re-extracted with 2 x 0.6 L of n-hexane.

The combined hexane phases are decolourized with 0.3% charcoal, dried over Na_2SO_4, then concentrated under vacuum at a temperature not above 40°C to an oil. 0.22 kg of a waxy extract are obtained, having an about 70% hyperforin content.

Example 2 - Synthesis of hyperforin acetate

A solution of 12 g of extract of plant obtained in Example 1 in 48 ml of pyridine is added with Ac_2O (9,8 mL) and stirred at room temperature. The reaction is checked by TLC (hexane-EtOAc 95:5; hyperforin Rf: 0.24; acetate Rf: 0.49). After 24 hours the reaction mixture is diluted with water and extracted with an hexane-ether mixture (3:1). The organic phase is washed with diluted HCl, saturated $NaHCO_3$ and saline solution. After drying

(Na_2SO_4) and evaporation, the residue is purified by chromatography on a silica gel column (*ca* 30 g), eluting first with petroleum ether to remove fats, then using hexane-EtOAc 95:5 as soon as the title compound starts to be eluted, 3,34 g (0.28%); hyperforin acetate is obtained as a colourless paste.

$C_{37}H_{54}O_5$, MW 578

CI-MS: 579 (M+H)+

IR (liquid film): 1779, 1732, 1660, 1634, 1447, 1377, 1339, 1146 cm^{-1}

^1H NMR (200 MHz, $CDCl_3$): 5.03 (br s, 2H), 5.00 (br s, 2H), 3.05 (dd, J=15, 7 Hz, 1H), 2.87 (dd, J=15, 7Hz, 1H), 2.22 (s, OAc), 1.66-1.53 (br s, 8 x 3H), 1.08 (d, J=6.5 Hz, 3H), 0.98 (s, 3H), 0.85 (d, J=6.5 Hz, 3H).

Example 3 - Synthesis of hyperforin 3,4,5-trimethoxybenzoate

A solution of plant extract of Example 1 (1.0 g) in pyridine (4 mL) is added with 3,4,5-trimethoxybenzoyl chloride (323 mg), and the solution is stirred at room temperature for 24 hours. The reaction cannot be checked by TLC, as the starting material and the product have a very close Rf value in different solvents. The reaction mixture is diluted with water and extracted with an ether-hexane mixture (3:1). The organic phase is washed with diluted HCl, saturated $NaHCO_3$ (washing with saline solution causes an emulsion to form). After drying (Na_2SO_4) and evaporation, the residue is purified by chromatography on a silica gel column (*ca* 5 g), eluting first with petroleum ether to remove fats, then with hexane-EtOAc 95:5 to obtain hyperforin trimethoxybenzoate (317 mg) as a colourless oil.

$C_{45}H_{62}O_8$, MW 730

CI-MS:731 (M + H)+

IR (liquid film): 1732, 1660, 1634, 1589, 1465, 1331, 1153, 1130. 914 cm^{-1}

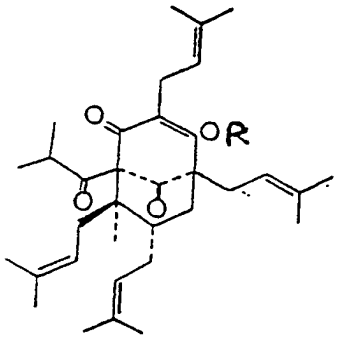
^1H NMR (200 MHz, $CDCl_3$): 7.27 (s, 2H), 5.04 (br S, 2H), 5.02 (br s, 2H), 3.86 (s, OMe), 3.82 (s, 2 x OMe), 3.10 (dd, J=15, 7 Hz, 1H), 2.92 (dd, J=15, 7 Hz, 1H), 1.66-1.53 (br s, 8 x 3H), 1.13 (d, J=6,5 Hz, 3H), 1.04 (s, 3H), 0.99 (d, J=6.5 Hz, 3H).

Example 4 - Coated tablets containing the product of Example 2

Hyperforin acetate	100 mg
Soy polysaccharides	18.25 mg
Cross-linked sodium carboxymethylcellulose	13.50 mg
Silica	6.50 mg
Polyvinylpyrrolidone	5.00 mg
Magnesium stearate	0.50 mg
Coating:	
Hydroxypropyl methylcellulose	3.75 mg
Talc	2.75 mg
Titanium dioxide	1.25 mg
Triacetin	0.75 mg
Polysorbate 80	0.25 mg
Red iron oxide	1.00 mg

CLAIMS

1. Compounds of formula I



in which R is:

- a saturated or unsaturated, straight or branched, C_1-C_{22} acyl group, optionally having one or more substituents, which can be the same or different, selected from halogen atoms, nitro, amino, C_1-C_6-alkylamino, di-C_1-C_6-alkylamino, C_1-C_6-acylamino groups;
- a cycloaliphatic or aromatic acyl residue in which the aromatic moiety optionally has one or more substituents, which can be the same or different, selected from halogen atoms, hydroxy, methoxy, amino groups;
- a glycidic residue in which one or more hydroxy groups are optionally alkylated or acylated.

2. Compounds as claimed in claim 1 wherein R is selected from acetyl, monochloroacetyl, butyryl, γ-aminobutyryl, p-aminobenzyl, trimethoxybenxyl, trimethoxycinnamyl, ß-glucoside and ß-galactosyl.

3. Extract of Hypericum perforatum containing a compound of claims 1 or 2.

4. Extract as claimed in claim 3 obtainable by a process which comprises:

a) extracting Hypericum perforatum flowering tops with

supercritical CO_2;

b) solubilizing the lipophilic extract from step a) in aqueous methanol or acetonitrile and extracting it with aliphatic hydrocarbons;

c) diluting the hydrophilic phase with water and counter-extracting it with aliphatic hydrocarbons;

d) concentrating the lipophilic phase;

e) treating the concentrate from step b) with a reactive derivative of a RCOOH acid or of an ROH sugar, wherein R is as defined in claim 1.

5. Pharmaceutical compositions containing as active ingredient a compound of claims 1-2 or an extract of claims 3-4, in admixture with a suitable carrier.

6. Compounds of formula I as antidepressants.

INTERNATIONAL SEARCH REPORT

[redacted]ional Application No
PCT/EP 99/03880

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07C49/653 C07C49/753 A61K35/78

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07C A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BRONDZ, ILIA ET AL: "The relative stereochemistry of hyperforin" TETRAHEDRON LETTERS, vol. 23, no. 12, 1982, pages 1299-1300, XP002117275 Great Britain the whole document ---	1-6
X	WO 97 13489 A (SCHWABE WILLMAR GMBH & CO ;ERDELMEIER CLEMENS (DE); GRETHLEIN ECKH) 17 April 1997 (1997-04-17) cited in the application page 3 -page 4 ---	3,5
X	EP 0 599 307 A (SCHWABE WILLMAR GMBH & CO) 1 June 1994 (1994-06-01) page 4, line 45 -page 5, line 22; claim 1 -----	3,5

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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INTERNATIONAL SEARCH REPORT

[redacted]ation on patent family members

Inte[redacted]nal Application No
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